Journal of Medical Virology 51:90–100 (1997)

In Situ Immune Responses in Crohn's Disease: A Comparison With Acute and Persistent Measles Virus Infection

A.J. Wakefield[1]*, R. Sim[1], A.N. Akbar[2], R.E. Pounder[1], and A.P. Dhillon[1]

[1]*Inflammatory Bowel Disease Study Group, Royal Free Hospital School of Medicine, London, United Kingdom*
[2]*Department of Clinical Immunology, Royal Free Hospital School of Medicine, London, United Kingdom*

The implied aetiological association of measles virus with Crohn's disease would be supported by detection of an immune response to infected cells in affected tissues. This study sought to detect and characterise in situ immune responses to measles virus in both acutely and persistently infected tissues, and in particular, Crohn's granulomata. Tissue sections from patients with Crohn's disease (n = 17), tuberculosis (n = 9), acute intestinal ischaemia (n = 5), acute measles pneumonitis (n = 2), acute measles appendicitis (n = 1), subacute sclerosing panencephalitis (SSPE; n = 1), and measles inclusion body encephalitis (MIBE; n = 1), were examined. Single and double immunohistochemical labelling was performed to identify both cytotoxic lymphocytes (CD8, TIA, perforin, Leu 7, CD45RO, CD45RA) and macrophages (KP1). The relationship of these cells to measles infected cells was examined by double immunolabelling with anti-measles virus nucleoprotein antibody. In both acute measles appendicitis and SSPE, $CD8^+/TIA^+$ cytotoxic lymphocytes (CTL) targeted infected cells. In the cases of Crohn's disease (13/17), MIBE, fatal pneumonitis, and one tuberculous granuloma, that were positive for measles virus, infected cells appeared to be targeted by macrophages rather than CTL. CTL in both tuberculous and Crohn's granulomata were similar in their peripheral distribution, number, and phenotype. The data suggest that measles-specific CTL responses may be attenuated in Crohn's disease compared with acute measles appendicitis and SSPE, and secondly, that an abnormal macrophage response to persistent measles virus infection of the intestine may result in granulomatous inflammation. ***J Med Virol 51:90–100, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: Crohn's disease; measles virus; cytotoxic lymphocytes

INTRODUCTION

The granuloma of Crohn's disease is an early, angiocentric inflammatory response [Wakefield et al., 1989, 1991] and is likely to represent a transient tissue reaction, possibly to foreign antigen. Despite being most frequently submucosal in location, the majority of immunological studies of this condition have concentrated upon mucosal immune responses [Strober and James, 1986]. A potential disadvantage of this approach is that the inflamed mucosa may manifest a diversity of immune responses that are consequent upon secondary translocation of luminal antigen. In contrast, mural granulomata are more likely to represent a specific reaction to, what may be, a causative agent, especially when these are located in deep layers of the gut, remote from ulcerated sites. Recent studies from the UK [Wakefield et al., 1993; Lewin et al., 1995], Sweden [Ekbom et al., 1994], and Japan [Miyamoto, 1995], have implicated persistent measles virus infection as an aetiological agent in inflammatory bowel disease, and live attentuated measles vaccine has also be implicated in this context [Thompson et al., 1995].

Whereas cytotoxic T lymphocyte (CTL) responses are a feature of virtually all common acute virus infections, persistent virus infection may result from either a failure to induce an adequate CTL response (low zone tolerance), or following an exhaustion of specific cytotoxicity (high zone tolerance), during the primary infection [Moskophidis et al., 1993]. We hypothesise that Crohn's disease, in particular, may develop from a low zone tolerance with persistent infection, following either low dose or attenuated measles virus infection early in life [Ekbom et al., 1994; Thompson et al., 1995].

Ultrastructural studies of Crohn's disease have revealed apparent CTL adherence to, and polarisation of

*Correspondence to: Dr. A.J. Wakefield, FRCS, University Department of Medicine, Royal Free Hospital School of Medicine, Rowland Hill Street, Hampstead, London NW3 2PF, UK.

Accepted 29 April 1996

TABLE I.

Antibody	Supplier	Source	Pretreatment	Antibody dilution	Control tissue
CD8	John Radcliffe Hospital, Oxford, UK	Murine	10 min microwave (MW)	1/2	Tonsil
TIA	Coulter Luton, Beds, UK	Murine	10 min MW	1/500	Tonsil
Leu 7	Beckton Dickinson Cowley, Oxfordshire, UK	Murine	None	Neat	Tonsil
CD45RA	Immunology Royal Free	Murine	2 min MW	1/10	Tonsil
CD45RO	School of Medicine (RFSM)	Murine	2 min MW	1/200	Tonsil
Measles N protein	CAMR, Porton Down	Rabbit	10 min MW	1/1000	SSPE MIBE
Perforin	Immunology RFSM	Murine	10 min MW	1/50	Peripheral blood lymphocytes
KP1	Dako Ltd, High Wycombe, Bucks, UK	Murine	10 min MW	1/200	Tonsil

cytotoxic granules towards, microvascular endothelium in foci of granulomatous inflammation [Wakefield et al., 1993]. The granulomatous macrophage reaction, typical of Crohn's disease, appears to be associated intimately with this endothelial lesion. This suggests an abnormal CTL response that either induces or is superceded by a granulomatous reaction. A transient macrophage reaction is normal after successful destruction of virally infected cells by the immune system: a persistent, granulomatous reaction implies failure to eliminate infection.

In the context of measles virus disease, interesting comparisons are available in the form of both acutely and persistently infected tissues: persistent infections include subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis (MIBE). Both extremely rare conditions, SSPE is a sequel to early measles exposure in an otherwise immunocompetent child, whereas MIBE is a complication of persistent measles virus infection in an immunosuppressed individual.

The purpose of the present immunohistochemical study was to examine the Crohn's granuloma with the following aims: to identify the presence, and characterise the phenotype, of CTL in the lesion; to determine whether these CTL target measles virus infected cells within affected tissues; and finally, to compare and contrast the immunological response in different measles-associated, ischaemic, and granulomatous inflammations.

PATIENTS, MATERIALS, AND METHODS

Paraffin processed, 10% formalin-fixed tissues from 17 patients with Crohn's disease were studied. These represented consecutive cases from whom vascular perfusion-fixed material was available. In each case the diagnosis was established by standard clinical, radiological, and histological criteria. The mean age of patients was 36 years (range 22–60), 12 were female, and tissues comprised 9 ileal and 12 colonic specimens. Twelve cases were receiving corticosteroid therapy. One case, a female, developed chronic, bloody diarrhoea at the age of 15 months immediately following live-attenuated measles vaccination. She subsequently underwent total colectomy and ileostomy at 19 months of age. All patients with Crohn's disease were in clinical relapse at the time of surgery. For comparison, tissues from three acutely infected measles cases (one appendix, two post-mortem lung tissues) and two persistently infected cases (cerebral tissues: one SSPE, one MIBE) were studied.

Serial tissue sections from these cases were stained with haematoxylin and eosin, and immunohistochemically stained for the following: TIA and performin were used to identify cells bearing cytotoxic granules [Akbar et al., 1984; Liu et al., 1995]. Cytotoxic cells were distinguished by $CD8^+$ (cytotoxic T cells) and Leu 7^+ (subsets of Natural Killer [NK] cells) [Akbar et al., 1994] phenotyping. In addition, memory T cells and naive T cells were distinguised using CD45RO and CD45RA antibodies, respectively. Macrophages were identified using KP1, and measles virus using polyclonal rabbit serum raised against the measles virus nucleocapsid protein. The specificity of the measles antibody has been described in detail elsewhere [Lewin et al., 1995]. Sections were counterstained with Mayer's haematoxylin, unless indicated otherwise. In some sections counterstaining was omitted in order to enhance the clarity of double immunostaining in particular.

Tissues from five cases of Crohn's disease (four from tissues bearing granulomata and one exhibiting lymphocytic inflammation only) were examined by double immunohistochemical staining using the following combinations: CD8 with either TIA or perforin, as functional markers of the cytotoxic cells, and measles with either TIA or KP1 in order to determine the spatial relationships between specific immune cells and measles virus infected cells. Cytotoxic cells were subclassified further, as either memory or naive, by double immunolabeling with TIA and either CD45RA or CD45RO antibodies, respectively. Details of the antibodies including source, dilution, specificity controls, and optimal staining conditions, are given in Table I. Additional controls were included in order to establish the specificity of the immunostaining: for each antibody single im-

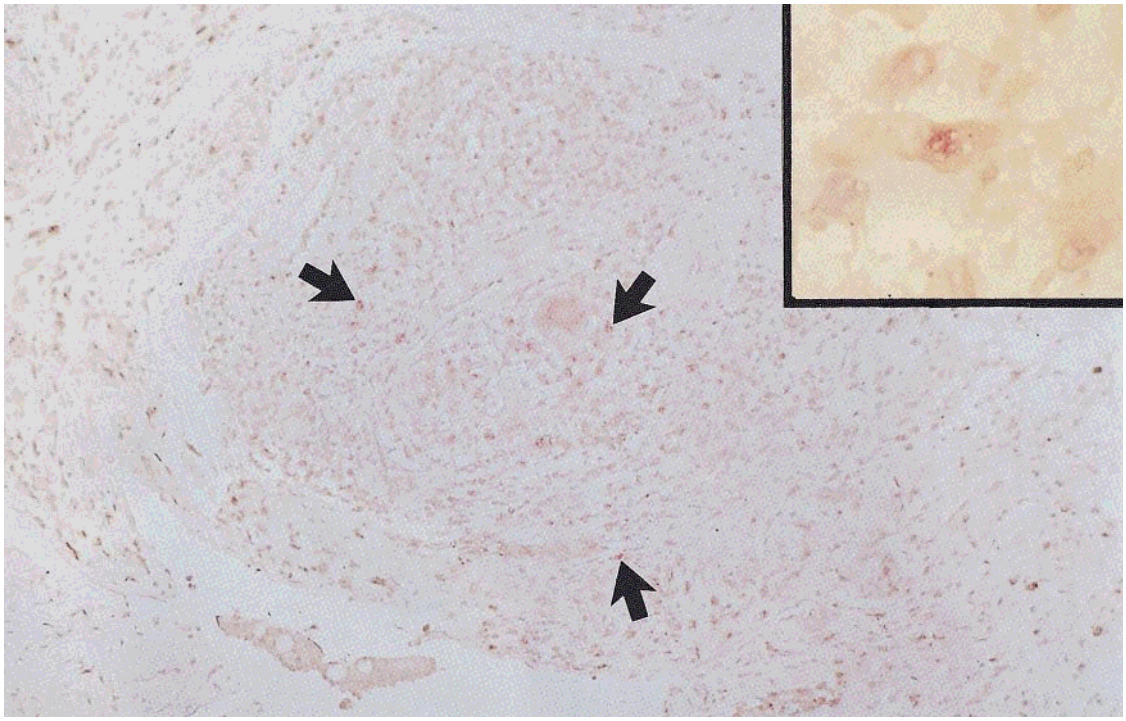

Fig. 1. A low power view of a serosal granuloma in Crohn's disease to show the distribution of immunostaining, and **(inset)** a high power view of the same lesion. Positively stained nuclei (arrowed) are confined to the granuloma (measles polyclonal antibody with no counterstain: original magnifications; a × 50, b × 1000).

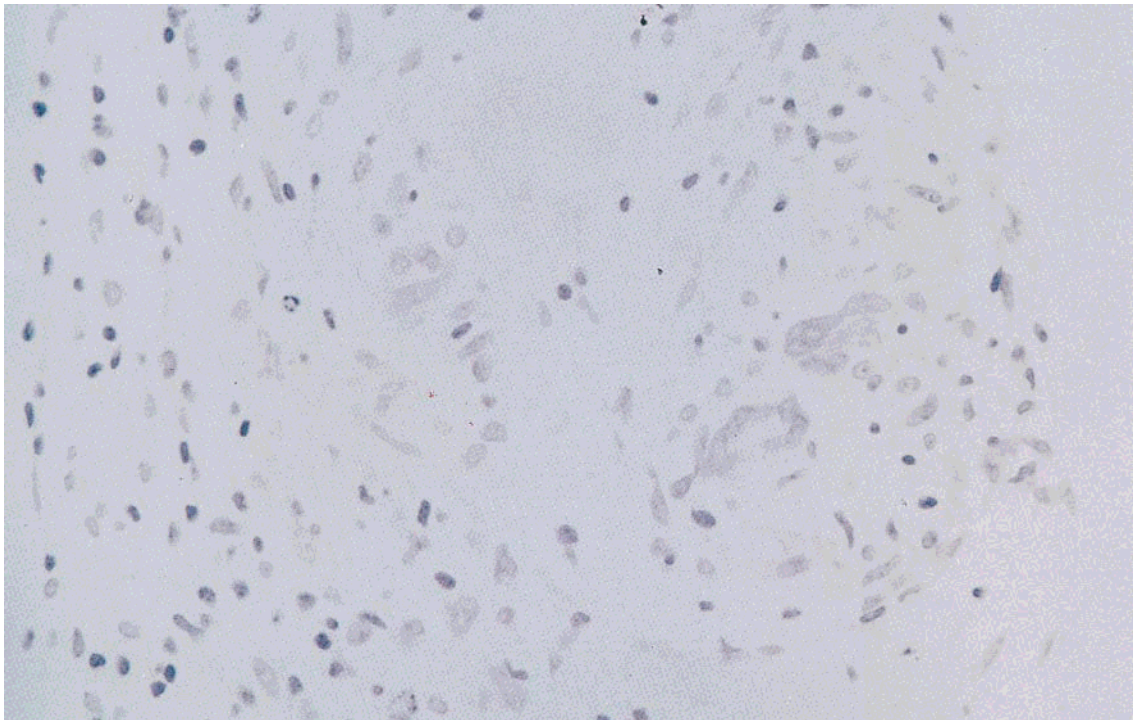

Fig. 2. A Crohn's granuloma processed with omission of the primary antibody; no staining is seen. A similar lack of staining was seen in all control tissue sections that were not treated with the primary antibodies, or that were treated with an irrelevant primary antibody (e.g., mumps nucleoprotein monoclonal antibody on Crohn's disease sections). (original magnification ×400.)

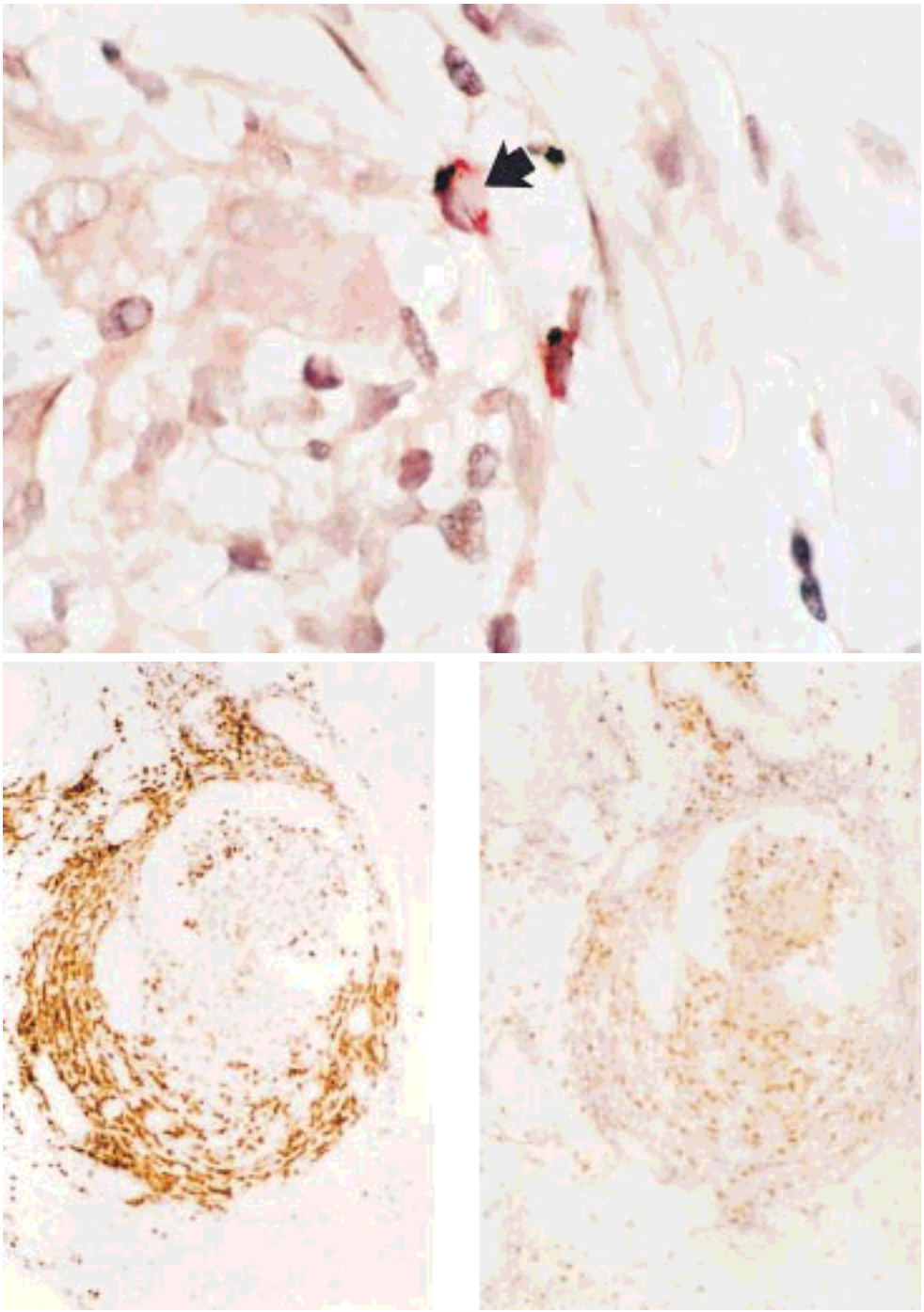

Fig. 3. A Crohn's granuloma that has been double-immunostained for CD8 (red) and TIA (brown) to identify cytotoxic T cells. Positive cells are shown at the periphery of the granuloma, one of which is in contact with the endothelial lining of the affected blood vessel (arrow) (original magnification ×1000).

Fig. 4. Serial sections of a Crohn's granuloma immunostained with anti-CD45RO **(left)** and anti-CD45RA **(right)** antibodies, respectively. Naive T cells are distributed around the periphery, while the centre of the granuloma contains the majority of memory T cells (original magnification ×50).

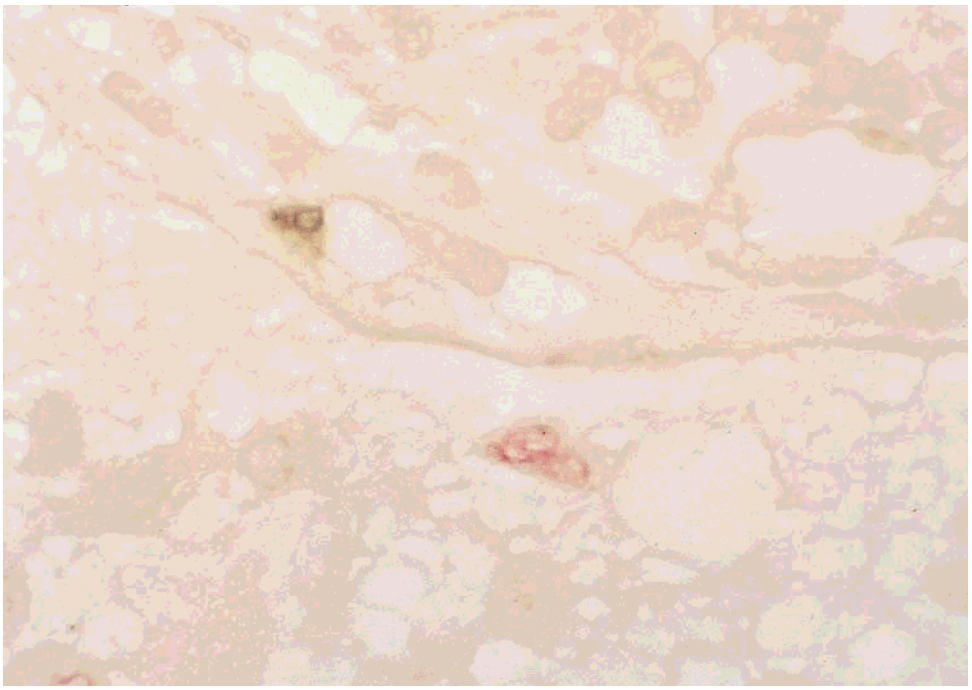

Fig. 5

Fig. 6

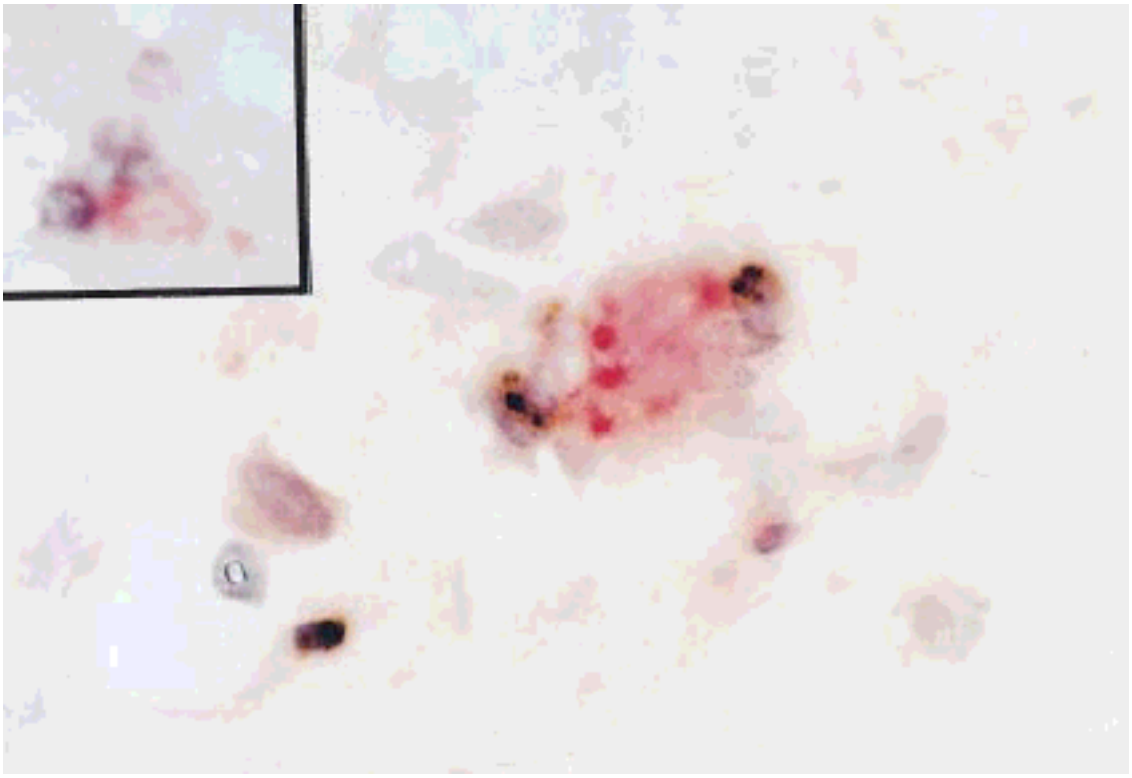

Fig. 7

Fig. 5. Colonic mucosal Crohn's disease from a child who underwent total colectomy at 19 months of age. A CTL (black) is seen in the mucosa, although exhibits no spatial proximity to measles virus infected cells (red) such as the endothelial-like cell at the edge of a thin walled mucosal vessel shown here (original magnification ×1000).

Fig. 6. Acute measles appendicitis double immunostained for TIA (black) and measles virus (red): micrograph shows small blood vessels adjacent to a focus of serosal vasculitis. Endothelial-like cells (closed arrow) and several cells adjacent to vessels are positive for measles virus (red). TIA^+ CTL (black) and measles virus infected cells are frequently apposed (open arrows). A single cell in the lower half of the micrograph (circled) appears to be both positive for measles virus and contain a TIA^+ granule suggesting that it may have been a target of CTL attack (no counterstain: original magnification ×800).

Fig. 7. Subacute sclerosing panencephalitis showing the close apposition of TIA^+ CTL (black) to a measled infected cell (red). **(inset):** A serial section of (7a) double immunostained for CD8 (blue) and measles virus (red), showing that CTL associated with measles virus infected cells are $CD8^+$ (original magnifications ×1000).

munostaining was used on sections of either tonsil, or persistently infected cerebral tissues in the case of measles primary antibody, in order to establish the staining pattern of the respective antibody. Thereafter, in double immunostained sections the order of applying the primary antibodies was reversed for each combination. This indicated that the signal (e.g., red or black) consistently localised to the appropriate target of the primary antibody. In addition, in separate experiments the second layer antibody was replaced by an irrelevant antibody, e.g., an antiepithelial antibody on brain sections, or a mumps primary antibody (Seralab, Crawley, Sussex, UK) on intestinal sections as described previously [Lewin et al., 1995] of the same class and source (e.g., murine): this indicated that, for each primary antibody, non-specific signal was not being detected at this stage of the procedure.

In order to examine the specificity of measles virus immunostaining for the Crohn's granulomata, nine cases of lymphadenomatous tuberculosis (TB) and five cases of acute intestinal ischaemia were also examined: all TB cases were positive by culture or Ziehl-Neelson staining. For the TB cases the mean age was 39 years (range 27–61), and four were female. Of the intestinal ischaemia cases, the mean age was 65 years (range 49–90), two were female, and specimens comprised three small intestinal and two colonic tissues. TB granulomata were also examined by immunohistochemistry for immuno-phenotype as described above, in order to determine whether the observed changes were disease specific, or characteristic of granulomatous inflammation in general. In order to quantify this, twenty-five consecutive granulomata were evaluated in both Crohn's disease and TB cases: cytotoxic cells within

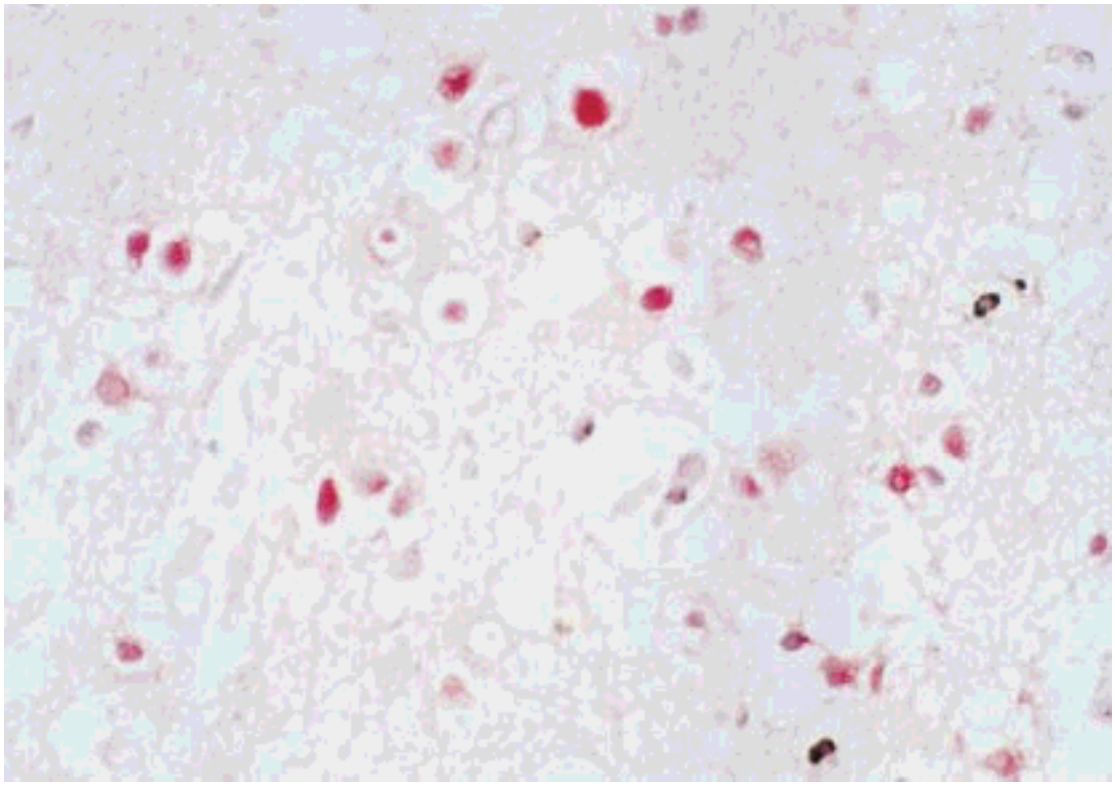

Fig. 8. Measles inclusion body encephalitis double immunostained for TIA (black) and measles virus (red). Staining for measles virus is evident in many cells, but few TIA^+ are seen, and these have no specific spatial relationship to measles virus infected cells (original magnification ×400).

granulomata bearing either TIA, perforin, or Leu 7 phenotype, were counted, and expressed as positive cells per medium power field (×40 objective). Twenty-five granulomata were examined in both Crohn's disease and tuberculous tissues.

RESULTS

Of the Crohn's disease cases, 13 of 17 contained granulomata in the sections studied: four exhibited lymphocytic inflammation only; a total of 75 granulomata were examined in the 13 granulomatous cases. Positive measles virus immunostaining was observed in 11 of 13 granulomatous cases and two of four non-granulomatous cases of Crohn's disease, and was present almost exclusively within granulomata or reactive lymphoid follicles, respectively (Fig. 1). Sections from acute measles appendicitis, acute measles pneumonitis, and SSPE tissues were all positive by measles immunostaining. In the appendix, positive staining was confined to Warthin-Finkeldey giant cells in lymphoid follicles and occasional foci or serosal or submucosal vasculitis. The appendix itself exhibited a chronic transmural inflammatory infiltrate with mucosal ulceration. In infected lung tissue, alveolar epithelium stained positively for measles virus nucleoprotein, as did syncytial giant cells which, together with a mixed inflammatory infiltrate, frequently filled alveolar acini. One of nine cases of TB exhibited positive measles immunostaining in macrophage-like cells within a granuloma: all remaining control tissues including sections of intestinal ischaemia were negative. Serial tissue sections from all disease groups treated identically, but omitting the primary measles antibody (Fig. 2) or applying an irrelevant primary antibody, were also negative.

Cytotoxic Cell Immunostaining

TIA^+ cells included both lymphocytes and neutrophils, which were readily distinguished morphologically. In Crohn's disease, cytotoxic lymphocytes were common in areas of granulomatous inflammation and were usually distributed around the periphery of the lesion, frequently adherent to the endothelium of involved vessels (Fig. 3). TIA^+ positive cells that were associated with granulomata numbered 6–50 (median 28) per medium power field (MPF). The numbers and distribution of these cells was similar in both Crohn's and TB granulomata.

$Perforin^+$ cells, readily identifiable in peripheral blood mononuclear cells, were far less abundant (1–2 per MPF) than TIA^+ cells in both Crohn's and TB granulomata (data not shown). Similarly, cells bearing the Leu 7 phenotype were rarely seen (1–4 per MPF; data not shown). Double immunostaining for either TIA or perforin and CD8 confirmed that the majority (> 95%) were $CD8^+$. A small percentage of TIA^+ cells were $CD8^-$

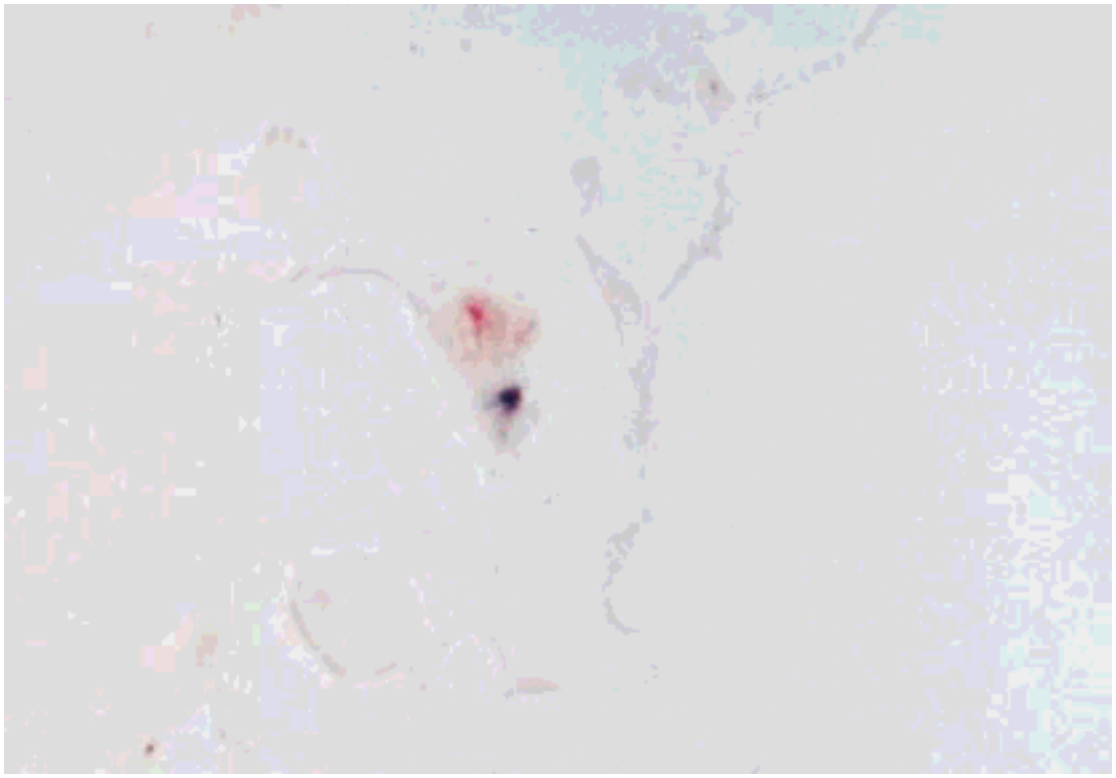

Fig. 9. Histologically normal mucosa in Crohn's disease, double immunostained with KP1 for macrophages (blue) and for measles virus (red): an isolated measles virus infected cell at the edge of a small blood vessel is closely apposed to a macrophage. No other macrophages are seen in this field of view (no counterstain; original magnification ×600).

(< 5%) but outnumbered Leu 7^+ cells in individual lesions. A distinctive pattern of $CD45RO^+$ and $CD45RA^+$ immunostaining was seen in Crohn's granulomata: lymphocytes that were interspersed with the central macrophage component of the lesion were $CD45RO^+$, whereas the majority of those around the periphery, were $CD45RO^-/CD45RA^+$ (Fig. 4). In sections that were double-immunostained for CTL and naive or memory phenotype, the majority (> 85%) of CTL, both within and surrounding granulomata, were $CD45RO^-$ (naive).

In acute measles appendicitis, CTL were not evident around Warthin-Finkeldey giant cells, but were common in foci of vascular inflammation, away from lymphoid follicles (see below). In MIBE tissue, CTL were rare and usually intravascular (see below), whereas in SSPE discrete clusters of $TIA^+/CD8^+$ lymphocytes were evident, either perivascularly or within the cerebral parenchyma (see below).

In double-immunostained sections of both the Crohn's granulomata, and a case of lymphocytic Crohn's disease that was positive for measles virus, there was no discernible spatial relationship between CTL and measles infected cells (Fig. 5). This was in contrast with the appearances in acute measles appendicitis (Fig. 6), and generally in the SSPE tissue (Fig. 7), in which CTL and measles infected cells were frequently, closely apposed. The apparent adherence of $TIA^+/CD8^+$ CTL to measles infected brain cells, including cerebral endothelium, was particularly apparent in SSPE where, otherwise, there was very little surrounding inflammation. Polarisation of cytotoxic granules towards measles infected cells was also apparent in the lesions of SSPE and acute measles appendicitis. In MIBE, where infection was associated with little or no lymphoid infiltrate, there was no evident spatial proximity of CTL to measles infected cells (Fig. 8). In acutely infected lung tissues, there were very few CTL, and these exhibited no relationship to measles infected cells (data not shown).

Macrophage Immunostaining

KP1 immunostaining confirmed the central role of the macrophage in granuloma formation. Apparently early, evolving granuloma had relatively few surrounding lymphocytes, but in more developed lesions the lymphocytic cuff was expanded (data not shown).

Sections of Crohn's disease, double immunostained for both macrophages and measles virus, exhibited features that were characteristic of all the cases studied by this technique. These included the close apposition of occasional isolated measles virus infected endothelial-like cells with individual macrophages (Fig. 9). More developed aggregates of macrophages were arranged in close proximity to discrete foci of measles infected cells (Fig. 10). In fully formed granulomata, positive immu-

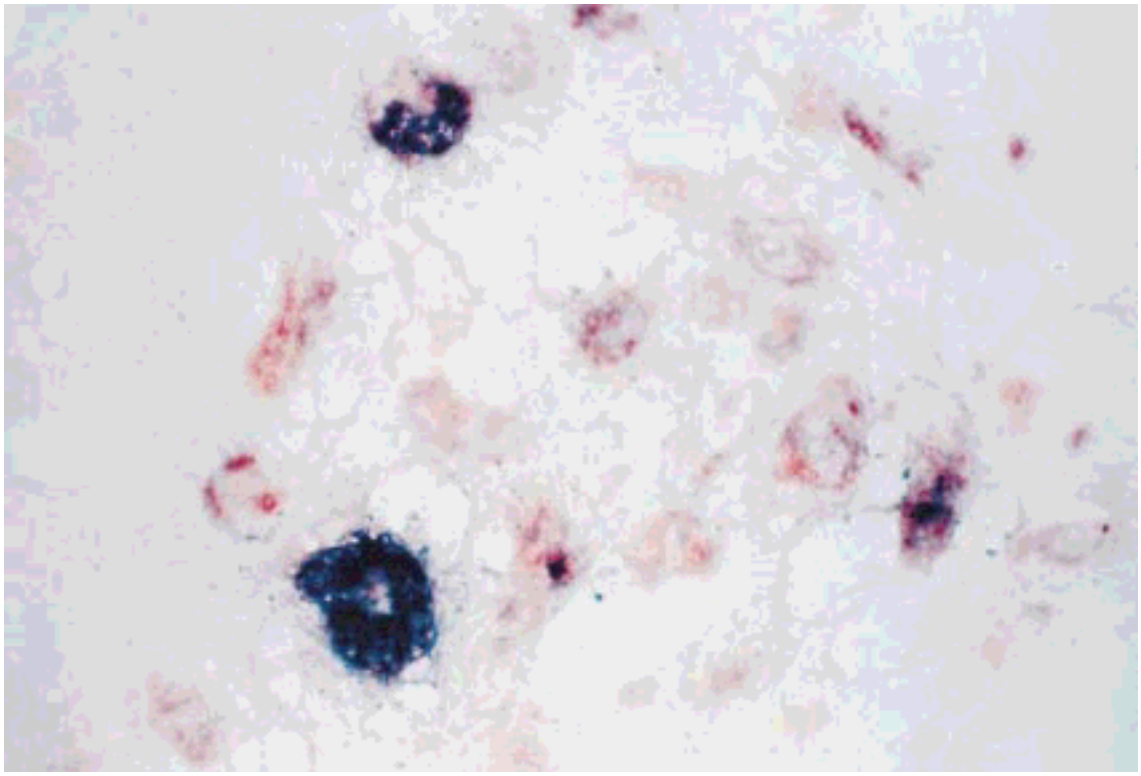

Fig. 10. A Crohn's disease microgranuloma double immunostained with KP1 (blue) and for measles virus (red). Measles virus immunostaining is seen at high power within several non-macrophage cells, and exhibits the same punctate pattern of staining seen in SSPE in Figure 7 (original magnification ×1000).

nohistochemical staining for measles virus was seen both within the nuclei of macrophages and in non-macrophage cells within the same lesion. A similar juxtaposition of measles infected cells and macrophages was seen in sections of MIBE (Fig. 11), but was rarely observed in the single cases of acute measles appendicitis and SSPE. However, double immunostaining for macrophages and measles virus in SSPE tissue revealed that measles infected cells were often dendritic microglia (cerebral macrophages) (data not shown). In acute, fatal measles pneumonitis the predominant immune cell was the macrophage: syncytial giant cells that stained positively for both KP1 and measles virus were abundant in the acini of these tissues (data not shown).

DISCUSSION

This paper presents some novel observations on the immunological phenotype of the Crohn's granuloma, particularly in relation to the cytotoxic component of this reaction. In addition, the data may reflect some of the differences in immune responses that distinguish acute and persistent measles virus infections in different tissues.

The differences between these two distinct states, acute and persistent infection, are likely to be complex, and reflect a number of host- and virus-related factors, including age and dose of exposure, virus strain, genotype, and immune status of the host, and the tissue that is infected. The resulting immunophenotype is also likely to be influenced by the fact that measles virus is, itself, profoundly immunosuppressive during the acute infection [von Pirquet, 1908; Cooradia et al., 1978; Pelton et al., 1982; Dagan et al., 1987]. The paucity of CTL in the cases of fatal pneumonitis may be an example of this phenomenon. CTL were abundant in the clinically milder measles appendicitis and were frequently juxtaposed to measles infected cells, a feature that was also characteristic of SSPE. albeit that only a single case was available for examination. In contrast, the data for Crohn's disease, MIBE, and acute measles pneumonitis suggest an intimate relationship between the macrophage and measles virus infected cells. Crohn's disease was distinguished from the other two conditions by the abundance of CTL in lesions, although these appeared to have no specific relationship to measles virus infected cells. The absence of an obvious CTL/infected cell interaction in Crohn's disease tissues does not exclude either a brief or occasional such event: however, both its demonstration in the single case of SSPE and reports of a similar interaction in hepatitis C-infected liver [Savage et al., 1995], suggest that measles-specific CTL responses may be attenuated in Crohn's disease.

Impaired immunoresponsiveness in the host, either as a general phenomenon in MIBE and possibly, fulminant pneumonitis, or perhaps specifically for measles virus in Crohn's disease, may predispose to a macro-

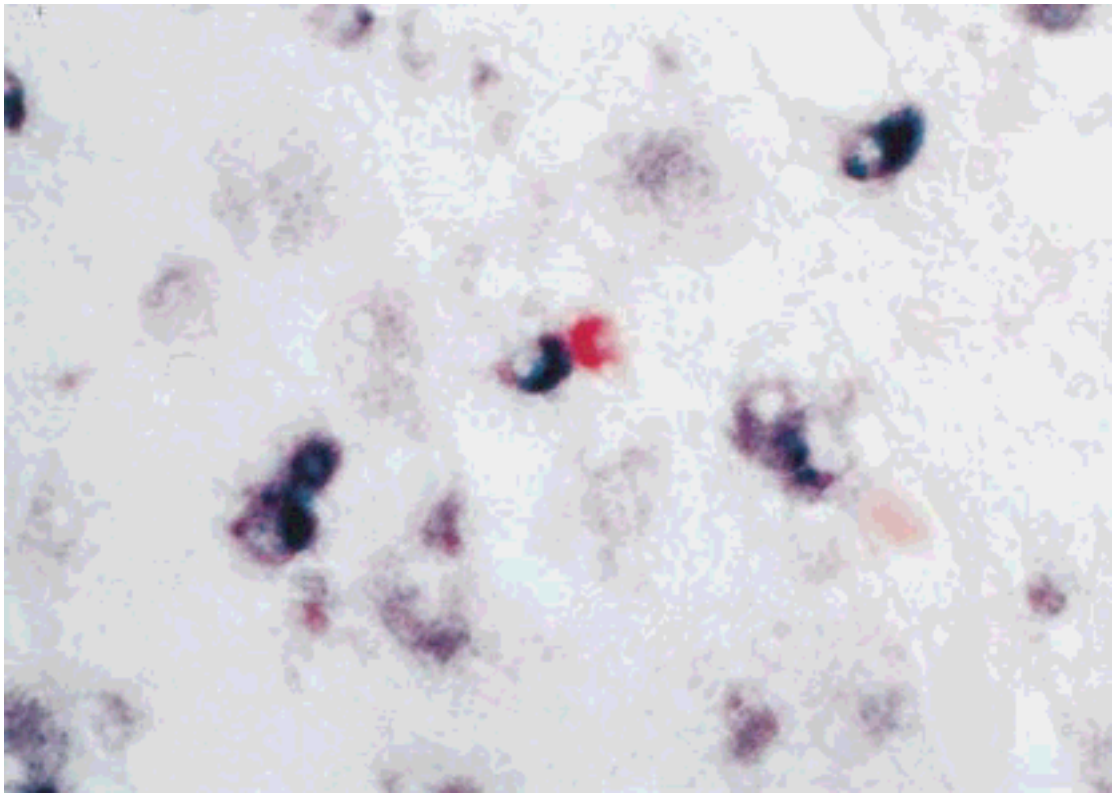

Fig. 11. Measles inclusion body encephalitis double immunostained with KP1 (blue) and for measles virus (red). A macrophage is closely apposed to a measles virus infected cell (original magnification ×1000).

phage response, rather than invoking specific cytotoxic immunity.

Immunodeficiency and failure to eliminate intracellular pathogens is known to be associated with chronic granulomatous inflammation [Bunch, 1983]. The presence of measles virus immunostaining in macrophages in both Crohn's disease and SSPE, raises the possibility of an additional, and novel pathogenic mechanism causing immune deficiency. Chronically infected macrophages could be functionally compromised, permitting virus persistence, as has been proposed for HIV infection [Belistio et al., 1984; Gartner et al., 1986; Boylston and Francis, 1988].

Steroid therapy did not appear to influence the immuno-phenotype in Crohn's disease, although a larger comparative study is required to clarify this issue. Albeit that apposition of macrophages and measles virus infected cells was observed in patients not receiving steroids, it would be reasonable to assume that immunosuppressive therapy might attenuate specific anti-measles virus cytotoxicity in Crohn's disease. In tuberculous tissues, no conclusions could be drawn about the relationship between *Mycobacterium tuberculosis* and specific immune cells, firstly, because of the paucity of acid fast organisms in the sections, and secondly, because the organisms were often located in areas of caseous necrosis.

Histological "snap shots" of such complex diseases should not be over interpreted. Nevertheless, some potentially interesting observations have emerged: firstly, the detection of measles virus, in non-immune, resident intestinal cells, some of which are certainly endothelium, suggests primary persistent infection of the intestine, rather than an influx of infected mononuclear cells from the circulation, secondary to inflammation from other causes. This observation supports previous studies [Wakefield et al., 1993; Lewin et al., 1995]. Secondly, in intestinal tissues, with the exceptions of acute measles appendicitis and the single case of TB, positive measles immunostaining was only seen in Crohn's disease.

In measles virus infected tissues, including Crohn's disease, a small subset of CTL were $CD8^-$: there is evidence that measles-specific cytotoxicity involves both MHC class I restricted $CD8^+$ T cells, and MHC class II restricted $CD4^+$ T cells [Jacobsen et al., 1984, 1987; Nanan et al., 1995]. It is possible, therefore, that $CD4^+$ CTL may also be important in the immune response to persistent measles virus infection. Unfortunately we were unable to identify an anti-CD4 monoclonal antibody that worked in formalin-fixed, paraffin processed tissue sections. Likewise, Leu 7 identifies only a subset of NK cells, and CD16 immunostaining for NK cells, feasible only in cryostat sections, would have discriminated cytotoxic cell populations more precisely. These issues will be examined in future studies.

In immunocompetent individuals, dose of virus exposure is perhaps the major factor in determining the outcome from acute measles infection [Aaby et al., 1984]. SSPE generally follows severe infection in an infant, and the epidemiological features of the disease, over-representation in males, rural populations, overcrowded homes, and developing areas of the world [Aaby et al., 1984], and a birth order effect such that lower birth order children are at greater risk, [Miller et al., 1992], are consistent with an initial state of tolerance following high dose infection. In contrast, these epidemiological features of SSPE are the direct opposite of those of Crohn's disease, supporting the hypothesis that, if causally related to Crohn's disease, low zone tolerance to either low dose or attenuated measles virus infection, may be the precursor of this conditions. Alternatively, intracellular sequestration of viral antigen which is subsequently expressed in association with MHC molecules and co-stimulatory signals during the course of, for example, an intercurrent infection may lead to T-cell activation/proliferation, and progression to clinical disease. Intercurrent infections are a well recognised trigger of both SSPE and inflammatory bowel disease [Kangro et al., 1990; Miller et al., 1992]. The testing of such hypotheses would be assisted greatly, by the development of an appropriate animal model.

In summary, this immunohistochemical study of in situ immune responses to measles virus confirms that, in Crohn's disease, the macrophage is a central component of the cellular immune reaction. For persistent measles virus infection, the resulting immuno-phenotype may reflect the circumstances in which the persistent state was established, for example, high or low dose exposure, and the immune status of the host. In Crohn's disease, the granulomatous macrophage reaction may be a response to persistent measles virus infection of the intestinal microvasculature.

ACKNOWLEDGMENTS

We are grateful to Francis Moll for his continued support and expertise, to Nilanthe Kelsey for help in preparation of the manuscript, and to Dr. Banu Anlar, Hacepte Hospital, Ankara, Turkey for provision of SSPE tissue. Our thanks are due, also, to Jackie Cordell, John Radcliffe Infirmary, Oxford, for the CD8 monoclonal antibody.

REFERENCES

Aaby P, Bukh J, Lisse IM, Smits AJ (1984): Risk factors in subacute sclerosing panencephalitis: Age- and sex-dependent host reactions or intensive exposure? Reviews of Infectious Diseases 6:239–250.

Akbar AN, Savill J, Gombert W, Bofill M, Borthwick NJ, Whitelaw F, Grundy J, Janossy G, Salmon M (1994): The specific recognition by macrophages of $CD8^+$, $CD45RO^+$ T cell undergoing apoptosis: a mechanism for T cell clearance during resolution of viral infections. Journal of Experimental Medicine 180:1943–1947

Belisito DV, Sanchez MR, Baer RL, Valentine F, Thorbecke GJ (1984): New England Journal of Medicine 310:1279–1282.

Boylston AW, Francis ND (1988): Does it matter which cells are infected by the human immunodeficiency virus type I? Journal of Pathology 156:93–96.

Bunch C (1983): Leucocytes in health and disease. In Wetherall DJ, Ledingham JGG, Warrell DA (eds): "Oxford Textbook of Medicine," Oxford University Press. (1978): pp 19.86–19.88

Cooradia HM, Wesley A, Henderson LG, Brain P, Vos GH, Hallet AF (1978): Alteration in immune responsiveness in acute measles and chronic post-measles chest disease. International Archives of Allergy and Applied Immunology 56:14–23.

Dagan R, Phillip M, Sarov I, Skibin A, Epstein S, Kuperman O (1987): Cellular immunity and T-lymphocyte subsets in young children with acute measles. Journal of Medical Virology 22:175–182

Ekbom A, Wakefield AJ, Zack M, Adami HO (1994): Perinatal measles infection and subsequent Crohn's disease. Lancet 344:508–510.

Gartner S, Markovits P, Markovitz DM, Kaplan M, Gallo RC, Popovic M (1986): The role of mononuclear phagocytes in HTL VIII/LAV infection. Science 233:215–219.

Jacobsen S, Richert JR, Biddison WE (1984): Measles virus-specific $T4^+$ human cytotoxic T cell clones are restricted by class II HLA antigens. Journal of Immunology 133:754–757.

Jacobsen S, Rose JW, Flerlage ML (1988): Induction of measles virus-specific cytotoxic T cells by purified measles nucleocapsid and haemagglutinin polypeptides. Viral Immunology 1:153–162.

Kangro HO, Chong SKF, Hardiman A, Heath RB, Walker-Smith JA (1990): A prospective study of viral and mycoplasma infections in chronic inflammatory bowel disease. Gastroenterology 98:549–553.

Lewin J, Dhillon AP, Sim R, Mazure G, Pounder RE, Wakefield AJ (1995): Persistent measles virus infection of the intestine: Confirmation by immunogold electron microscopy. Gut 36:564–569.

Liu CC, Walsh C, Young JDE (1995): Perforin: Structure and function. Immunology Today 16:194–201.

Miller C, Farringdon CP, Harbert K (1992): The epidemiology of subacute sclerosing penencephalitis in England and Wales 1970–1989. International Journal of Epidemiology 21:998–1119.

Miyamoto H, Tanaka T, Kitamoto N, Fukada Y, Shimoyama T (1995): Detection of immunoreactive antigen with a monoclonal antibody to measles virus in tissue from patients with Crohn's disease. Journal of Gastroenterology 30:28–33.

Moskophidis D, Lechner F, Pircher H, Zinkernagel RM (1993): Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature 362:758–760.

Nanan R, Carstens C, Kreth HW (1995): Demonstration of virus-specific $CD8^+$ memory T cells in measles-seropositive individuals by in vitro peptide stimulation. Clinical and Experimental Immunology 102:40–45

Pelton BK, Hylton W, Denman AM (1982). Selective immunosuppressive effects of measles virus infection. Clinical and Experimental Immunology 47:19–26.

Savage K, Karaweznski K, El-Batanony M, El-Refaie AO, Khakoo S, Dusheiko G, Dhillon AP (1995): Detection of Hepatitis C viral antigen in frozen liver biopsies. Journal of Pathology 175:147A.

Strober W, James SP (1986): The immunological basis of inflammatory bowel disease. Journal of Clinical Immunology 6:415–432.

Thompson NP, Montgomery S, Pounder RE, Wakefield AJ (1995): Is measles vaccination a risk factor for inflammatory bowel disease? Lancet 345:1071–1074.

von Pirquet (1908): Das verhalten der kutanen tuberculin reaktion wahrend der masern. Deutche Medicine Wochenschrift 341: 1297–1300.

Wakefield AJ, Sawyerr AM, Dhillon AP, Pittilo RM, Rowles PM, Lewis AAM, Pounder RE (1989): Pathogenesis of Crohn's disease: Multifocal gastrointestinal infarction. Lancet i:1057–1062.

Wakefield AJ, Sankey EA, Dhillon AP, Sim R, Hudson M, Pounder RE (1991): Granulomatous vasculitis in Crohn's disease. Gastroenterology 100:1279–1287

Wakefield AJ, Pittilo RM, Sim R, Cosby L, Stephenson JR, Dhillon AP, Pounder RE (1993): Evidence of persistent measles virus infection in Crohn's disease. Journal of Medical Virology 39:344–353.